Analysis of α-Branched Fatty Acids by Gas Chromatography and Mass Spectrometry[1]

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ABSTRACT

Gas liquid chromatography and mass spectrometry were utilized in combination to identify isomeric α-branched chain fatty acid methyl esters. In a given isomeric series equivalent chain length, values decreased with increase in the number and size of α-alkyl substituents. Mass spectra of the α-monoalkyl derivatives are characterized by prominent McLafferty rearrangement ion peaks, whereas those of the α,α-dialkyl isomers contain the former ions plus an α-cleavage ion.

INTRODUCTION

In previous reports from this laboratory, we have described procedures for the conversion of normal chain fatty acids to their isomeric α-branched chain counterparts (1,2). The products were mixtures of isomeric branched chain acids, comprised of either α-monosubstituted (dialkylacetic acids) or α,α-disubstituted (trialkylacetic acids) moieties. Methods were therefore required which could distinguish between isomeric branched chain products. Whereas gas liquid chromatography (GLC) has been used extensively for the compositional analysis of normal chain fatty acid mixtures (3,4), this method has had limited success when applied to branched chain fatty acid mixtures (5,6). On the other hand, mass spectrometry has been used with considerable success for the location of alkyl branches in fatty acid esters (7-9). The mass spectra of α-branched fatty acids are different from those of the straight chain isomers, but the processes leading to both types of spectra are similar (10). Knowledge of the fragmentation paths leading to these spectra permits identification of the branched acid isomers. However, analysis of the spectra of branched chain acids does not always permit a definitive conclusion regarding the number of alpha branches. It is the purpose of this communication to report a method by which dialkyl and trialkylacetic acids can be distinguished with the aid of a newly observed fragmentation pattern of trialkylacetic acids.

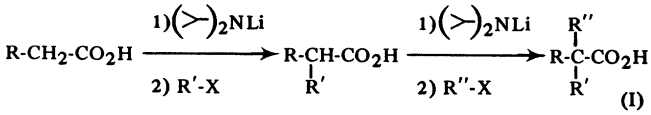
EXPERIMENTAL PROCEDURES

The mass spectra were obtained on a Dupont 21-492 double focusing instrument operating at an ionization potential of 70 eV. The samples were introduced by either a batch inlet system or by direct probe, depending on the sample volatility. The gas chromatograph was a Hewlett Packard 7620A instrument equipped with stainless steel 1/8 in. x 10 ft columns containing 10% UCW-98 on Chromosorb W, 80-100 mesh (column A) or 10% diethyleneglycol succinate on Gas-Chrom S, 60-80 mesh (column B). The carrier gas flow for both columns was 40 ml helium/min. The columns (used for the separation of compounds 1-13 shown in Table I) were operated in the isothermal mode under the following conditions: methyl esters *1-4*, A column 95 C, B column 100 C; methyl esters *5-13*, A column 170 C, B column 200 C. Equivalent chain length (ECL) values were obtained by the graphic method using a plot of logarithm of retention time against the ECL of standard fatty acid methyl esters (4).

Isomerically pure α-branched fatty acids were prepared from normal chain fatty acids by the α-anion procedure described by Pfeffer et al. (11). The methyl esters were prepared by reaction of the acids with diazomethane. All the acids and methyl esters had infrared and proton magnetic spectra consistent with their assigned structures. All new compounds gave satisfactory elemental analyses.

DISCUSSION

The model α-branched chain fatty acids prepared in this study were obtained from normal chain fatty acids by the α-anion technique developed by Pfeffer et al. (11), equation I, and are listed in Table I.



(I)

Several series of isomeric acids were investigated, ranging in carbon number of the acid from C_9 to C_{18}. Table I lists the ECL values (4) for the methyl esters of the α-branched acids on a polar (DEGS) and nonpolar (UCW-98) stationary phase. As expected, the α-branched isomers have lower ECL values than the corresponding straight chain compounds. This decrease in ECL is much more pronounced on the polar stationary phase than on the nonpolar phase. The decrease on the polar column varied from 1.8 to 2.5 units whereas, with the nonpolar column, the decrease in ECL ranged from 0.9 to 2.2 units. A comparison of ECL values of isomeric dialkyl and trialkylacetic acids indicates that the latter have consistently lower values (compare esters *2, 3; 6, 7;* and *11, 12).* However, this generalization is valid only when the α-alkyl branches are of comparable chain length. In addition, for any given pair of isomeric dialkyl or trialkylacetic acids, the larger the α-alkyl substituent, the lower the ECL. For example, for the isomeric C_{18} methyl esters *10* and *11*, the α-butyl isomer *11* has an ECL of 0.5 carbon atom lower on both columns than the corresponding α-ethyl isomer *10*. With the isomeric C_{17} acids *8* (α-methyl) and *9* (α-heptyl), this difference in ECL is even more apparent. The same general principle applies, but to a lesser degree, to the isomeric trialkylacetic acids (compare entries *3, 4* and *12, 13).* From the above results, it is apparent the GLC by itself does not allow for the differentiation of isomeric dialkyl and trialkylacetic acids.

However, a conclusion as to the true structure of an α-branched chain fatty acid can be made from a comparison of the mass spectral fragmentation ions of its methyl ester. Aside from those ions which are characteristic of paraffinic and olefinic compounds, the mass spectra of the branched

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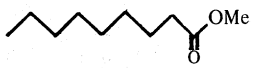
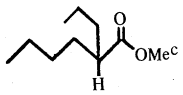
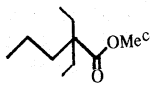
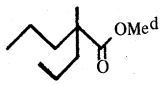
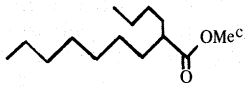
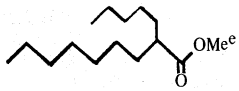
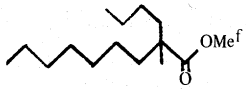
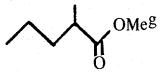
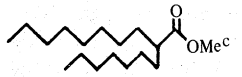
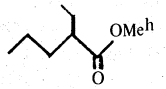
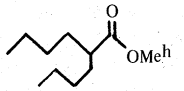
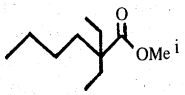
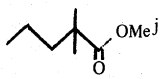
TABLE I

Gas Liquid Chromatography-Mass Spectrometry Data for Isomeric α-Branched Methyl Esters

Compound number	Structure	ECL[a] UCW-98	ECL[a] DEGS[b]	M^+ m/e	M^+ %	$[M-C_nH_{2n}]^+$ m/e	$[M-C_nH_{2n}]^+$ %	$[M-59]^+$ m/e	$[M-59]^+$ %
1		9.0	9.0	172	3	74	100	113	<1
2		8.1	7.2	172	<1	130 116	56 55	113	15
3		7.8	6.9	172	<1	144 130	20 54	113	65
4		7.7	6.7	172	<1	130	100	113	62
5		11.7	10.8	228	7	172 130	47 100	169	2
6		12.7	10.8	242	9	172 144	59 100	183	2
7		12.3	10.7	242	2	186 144	57 100	183	40
8	n-$C_{12}H_{25}$ 	16.5	15.8	284	30	88	100	225	1
9		15.8	14.8	284	20	186 172	89 100	225	2
10	n-$C_{12}H_{25}$ 	17.3	16.4	298	29	270 102	9 100	239	2
11	n-C_9H_{19} 	16.9	15.9	298	25	242 130	37 100	239	2
12	n-C_9H_{19} 	16.7	15.5	298	5	270 130	14 100	239	31
13	n-$C_{12}H_{25}$ 	16.8	15.6	298	5	102	100	239	16

[a]ECL = Equivalent chain length.

[b]DEGS = Diethyleneglycol succinate.

[c]See ref. 11.

[d]mp 44-44.5 C.

[e]bp 123-125 C (0.05 Torr).

[f]bp 108-111 C (0.07 Torr).

[g]mp 46-48 C.

[h]See ref. 13.

[i]mp 22-23 C.

[j]mp 45-46 C.

methyl esters are dominated by fragmentation at the carbomethoxy substituted carbon atom. Two types of cleavage processes predominate: simple cleavage at the α-position, resulting in a loss of the carbomethoxy group, to give a $[M-59]^+$ ion; and the McLafferty rearrangement (12) via an available γ-hydrogen atom to a $[M-C_nH_{2n}]^+$ ion (Table I). Substitution at the α-position by ethyl or larger alkyl groups provides two or three sets of γ-hydrogens and thus two or three routes for the McLafferty rearrangement (10). While all rearrangements will occur, participation of hydrogen from the larger alkyl group is favored (Table I, entries *10-13*). Successive substitution by methyl groups causes a shift of the McLafferty ion from m/e of 74, where it is found in normal chain methyl esters, to m/e of 88 and 102 for the monomethyl and dimethyl derivatives, respectively (Table I, entries *8* and *13*). However, a simple analysis of the above rearrangement ions does not readily discern whether the branched methyl ester in question is an isomeric dialkyl or trialkylacetic acid structure. This distinction can be made from an examination of the intensity of the cleavage ion $[M-59]^+$. It has been found in this study that cleavage at the α-carbon atom to give a $[M-59]^+$ is much more prominent in the spectra of trialkylacetic acid methyl esters than in the spectra of corresponding isomeric dialkylacetic acid methyl esters. For example, in the isomeric C_9 series (Table I, entries *1-4),* the intensity of the $[M-59]^+$ ion in the spectra of acids *3* and *4* is on the order of 60% of the base peak, whereas for acid *2* the ion is only 15% of the base peak. In the spectrum of the normal chain isomer *1* this $[M-59]^+$ ion is of very low magnitude. With the isomeric C_{14} acid pair *6* and *7*, the $[M-59]^+$ ion of the trialkylacetic acid *7* is some 20 times grater in intensity than that of the corresponding dialkylacetic acid *6*. Increasing the mol wt of the branched acids into the C_{17}-C_{18} range, acids *8-13,* results in increased intensity of the $[M-59]^+$ ion for tertiary acids *12* and *13,* whereas there is a concomitant decrease in the intensity of the M^+ ion. By contrast, the dialkyl acetic acids *8-11* give prominent M^+ ions and very minor $[M-59]^+$ ions. It is apparent, then, that cleavage at the α-carbon atom is very favorable in the case of trialkylacetic acids, inasmuch as the residual carbocation has a tertiary structure. Also, the increase in the intensity of the $[M-59]^+$ ion occurs at the expense of the intensity of the M^+ ion. The above conclusions, however, are valid only when comparing aliphatic acids and may not apply to branched aromatic carboxylic acids. In summary, the exact structure of an isomeric α-branched fatty methyl ester is deduced from determination of its ECL in conjunction with an analysis of its mass spectrum. Specifically, this is done by determining the relative intensity of its McLafferty rearrangement ions and the $[M-59]^+$ ion to the molecular ion.

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